

## Distribution of *Synechococcus* and *Prochlorococcus* in the central Adriatic Sea

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*The abundance of Synechococcus and Prochlorococcus were determined at twenty-one stations located along the eastern coast of the central and southern Adriatic and at three stations located in the open central Adriatic Sea. Synechococcus abundance ranged from  $10^2$  to  $10^5$  cells  $\text{mL}^{-1}$  in the coastal area, and from  $10^3$  to  $10^4$  cells  $\text{mL}^{-1}$  in the open sea. Prochlorococcus abundance in the coastal area ranged from 0 to  $10^4$  cells  $\text{mL}^{-1}$ , and from  $10^3$  to  $10^4$  cells  $\text{mL}^{-1}$  in the open sea. The seasonal distribution of Synechococcus and Prochlorococcus mostly showed an increase in abundance during the warmer period and a decrease during winter. The highest abundance of Synechococcus was found in Kaštela Bay with values at  $4.6 \times 10^5$  cells  $\text{mL}^{-1}$ , while the highest abundance of Prochlorococcus was determined in the Šibenik area and recorded as  $7.1 \times 10^4$  cells  $\text{mL}^{-1}$ . Synechococcus was found to be more abundant than Prochlorococcus in most cases. The abundance of Synechococcus was influenced more by temperature than nutrient availability. The abundance of Prochlorococcus was influenced by nutrient availability and the movement of water masses more than by HNF.*

**Key words:** *Synechococcus*, *Prochlorococcus*, Adriatic Sea

### INTRODUCTION

Photosynthetic picoplankton, represented primarily by the genera *Synechococcus* and *Prochlorococcus*, are major contributors of biomass and primary production in oligotrophic oceanic ecosystems (JOHNSON & SIEBURTH, 1979; WATERBURY *et al.*, 1979; PARTENSKY *et al.*, 1999a). In the oligotrophic Mediterranean Sea, picophytoplankton has a great contribution to total phytoplankton biomass and production (MAGAZZÙ & DECEMBRI, 1995; AGAWIN *et al.*, 2000). Photosynthetic picoplankton appears to be an essential component of microbial food webs and carbon flow, especially during the summer in coastal systems (KUOSA, 1991; WORDEN *et al.*, 2004).

Previous studies in the Adriatic Sea were mainly related to investigations of abundance

and the distribution of the genus *Synechococcus*. In the northern open sea and central areas of the Adriatic Sea, the abundance of *Synechococcus* ranged from  $10^2$  to  $10^6$  (VANUCCI *et al.*, 1994) and from  $10^3$  to  $10^5$  (NINČEVIĆ *et al.*, 2006), respectively. Studies of the genus *Prochlorococcus* in the Adriatic Sea are very scarce. Namely, only data for the abundance of this genus in the northern Adriatic are published (RADIĆ *et al.*, 2009).

In this study, we present the first detailed photosynthetic picoplankton data set available for the eastern coast of the central and southern Adriatic Sea and for the open central Adriatic. The goal of this paper was to study the seasonal cycle of *Synechococcus* and *Prochlorococcus* in the coastal and open Adriatic Sea, and to determine the ecological factors that affected them.

## MATERIAL AND METHODS

Water samples for *Synechococcus*, *Prochlorococcus* and nutrient analysis were taken at monthly intervals from January to December 2005. Samples were taken from 2 to 8 depths (0 - 260 m) between the surface and the near-bottom layer at twenty-one stations located along the eastern coast of the central and southern Adriatic and three stations (CA001, CA003, CA009) located in the open central Adriatic Sea (Fig.1). Niskin bottles (5 L) were used for sampling and samples were immediately processed on board. Temperature and salinity were also recorded using a SeaBird 25 CTD profiler. Nutrient concentrations ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , total dissolved inorganic nitrate-DIN and soluble reactive phosphate-SRP) were determined with the autoanalyser modified method by GRASSHOF (1976).

Abundances of *Synechococcus* and *Prochlorococcus* were determined using flow cytometry (MARIE *et al.*, 1997), and different populations were distinguished according to light diffraction, red emission of cellular chlorophyll content and orange emission of phycoerythrin-rich cells. Samples were preserved in 0.5% glutaraldehyde, frozen at  $-80^\circ\text{C}$  and stored until analysis. Samples were analysed on a Beckman Coulter EPICS XL-MCL with a high flow rate from 1 to  $1.2 \mu\text{L sec}^{-1}$ . Fluorescence beads were added to calibrate the cells' fluorescence intensity (Level-II Epics Division of Coulter Corporation Hialeah, Florida).

The number of heterotrophic nanoflagellates (HNF) was estimated using epifluorescence microscopy. Samples were stained with 4'-6-diamidino-2-phenylindole (DAPI) for 10 minutes and were filtered through  $0.8 \mu\text{m}$  pore

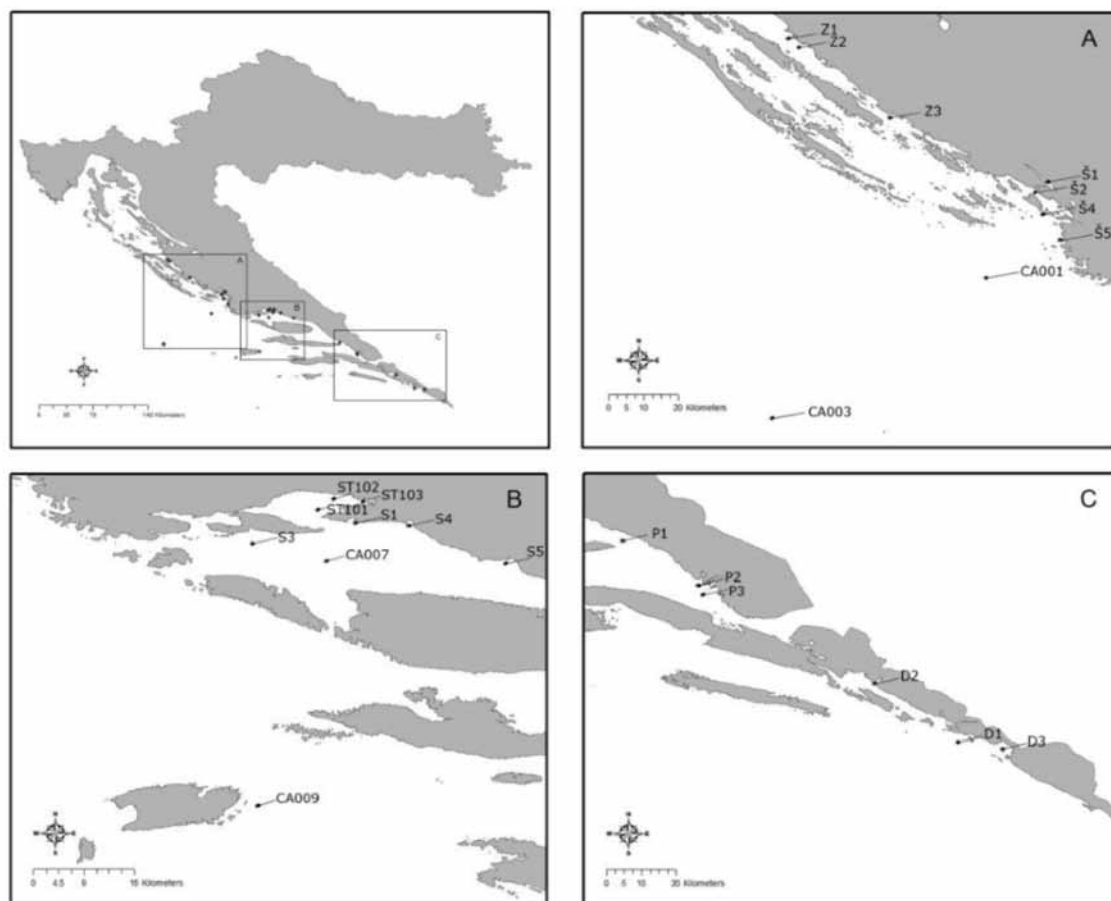


Fig. 1. Study area with sampling stations. A) Coastal areas: Zadar (Z1-Z3), Šibenik (Š1-Š5) open sea areas (CA, CA003); B) coastal areas: Kaštela Bay (ST101-ST103), Split (S1-S5, CA007) open sea areas (CA009); C) coastal areas: Ploče (P1-P3), Dubrovnik (D1-D3)

diameter polycarbonate filters (Millipore, Ireland). Microscope slides were observed with an Olympus microscope under UV light illumination at a magnification of 1,000X (PORTER & FEIG, 1980). Relationships between abiotic and biotic factors, and the abundance of *Synechococcus* and *Prochlorococcus* were determined using the Pearson rank correlation index.

## RESULTS AND DISCUSSION

### Physicochemical conditions of seawater

At coastal stations, thermal stratification of the water column began in May and lasted until September when the isothermal period began. The lowest values and widest ranges of temperature and salinity were recorded in the areas of Šibenik, Kaštela Bay, Split and Ploče, mostly due to the influence of the river Krka followed by the rivers Jadro, Žrnovnica, Cetina and Neretva (Table 1). At the open sea stations CA001 and CA003, thermal stratification was noted from June to September and temperature profiles showed thermal stratification in the upper 100 metres. Unlike the aforementioned

two open sea stations, thermal stratification of the water column began earlier in April and lasted longer until November at the open sea station CA009. During the investigation period at open sea stations, the temperature varied from a minimum of 10.4°C to a maximum of 24.0°C, while salinity varied from 36.4 to 38.9 (Table 1).

Along the coastal sea, average values of nitrates, nitrites and phosphates were uniform during the isothermal and stratified period, while concentration of ammonium increased during the stratified period in relation to the isothermal period (Table 2). At the open sea stations, concentrations of nitrates, nitrites and ammonia were higher during the isothermal period than the stratified period. Comparing the values of phosphates at the open sea stations, a higher concentration was determined during the stratified period in relation to the isothermal period (Table 2).

### Abundance of *Synechococcus*

Average monthly abundances of *Synechococcus* in the central and southern coastal area ranged from  $1.3 \times 10^2$  to  $1.6 \times 10^5$  cells mL<sup>-1</sup>.

Table 1. Ranges of temperature (°C) and salinity in coastal and open sea areas

Isothermal period Temperature			Stratified period Temperature	
Area	Minimum	Maximum	Minimum	Maximum
Zadar	11.22	20.94	15.18	24.61
Šibenik	13.09	22.22	6.33	22.86
Kaštela Bay	9.10	22.56	9.45	26.93
Split	12.77	18.22	11.73	24.16
Ploče	12.59	22.81	16.39	23.65
Dubrovnik	14.93	24.48	14.77	24.48
open sea	11.50	19.39	10.38	23.96
Salinity			Salinity	
Zadar	37.67	38.81	37.47	38.51
Šibenik	26.37	38.80	4.42	38.79
Kaštela Bay	31.94	38.37	34.12	38.87
Split	23.20	38.54	29.70	38.66
Ploče	26.47	38.62	31.40	38.46
Dubrovnik	31.79	38.82	30.23	38.87
open sea	35.72	38.85	36.37	38.94

Table 2. Ranges of abiotic and biotic factors measured

Complete dataset	Nitrate	Nitrite	Ammonia	DIN	SRP
	$\mu\text{molL}^{-1}$	$\mu\text{molL}^{-1}$	$\mu\text{molL}^{-1}$	$\mu\text{molL}^{-1}$	$\mu\text{molL}^{-1}$
Coastal sea					
<i>isothermal period</i>					
Minimum	0	0	0.08	0.36	0
Maximum	17.67	0.95	6.87	18.26	1.50
Arithmetic Mean	1.16	0.14	0.99	2.30	0.06
<i>stratified period</i>					
Minimum	0	0	0.04	0.34	0
Maximum	53.86	1.05	27.30	57.93	1.50
Arithmetic Mean	1.33	0.10	1.53	2.96	0.06
Open sea					
<i>isothermal period</i>					
Minimum	0.31	0.03	0.37	0.92	0
Maximum	4.20	0.44	2.05	4.60	0.06
Arithmetic Mean	1.19	0.18	0.99	2.35	0.03
<i>stratified period</i>					
Minimum	0	0	0.04	0.39	0
Maximum	3.11	0.28	2.28	4.45	0.28
Arithmetic Mean	0.61	0.08	0.72	1.41	0.06

The average monthly abundances of *Synechococcus* in the open sea ranged from  $3.09 \times 10^3$  to  $6.3 \times 10^4$  cells  $\text{mL}^{-1}$ . Variations in the abundances of *Synechococcus* were more pronounced in the coastal sea areas compared to the open sea area (Fig.2 to Fig.8).

Comparing all investigated areas of various depths to the values of the surface layer, the highest individual number of *Synechococcus* was found in Kaštela Bay (ST 101) and was recorded as  $4.6 \times 10^5$  cells  $\text{mL}^{-1}$  (Fig. 9). Abundances of *Synechococcus*, determined in the range from  $10^2$  to  $10^5$  cells  $\text{mL}^{-1}$ , are consistent with previous results reported by NINČEVIĆ GLADAN *et al.* (2006) concerning *Synechococcus* abundance at stations ST101 and CA009. According to the literature, similar ranges of *Synechococcus* abundance ( $10^3$  to  $10^5$  cells  $\text{mL}^{-1}$ ) have been found in the north-western Mediterranean (VAQUER *et al.*, 1996; BERNARDI AUBRY *et al.*, 2006), eastern Mediterranean (LI *et al.*, 1993; UYSAL &

KÖKSALAN, 2006) and the northern Adriatic Sea (PAOLI & DEL NEGRO, 2006; RADIC *et al.*, 2009).

Our investigations revealed the presence of *Synechococcus* over a wide temperature range in the coastal area as well as in the open sea (Table 1). Moreover, increased numbers of *Synechococcus* were found during the warmer seasons, except in the area of Šibenik, Kaštela Bay and Ploče, where high values were observed during the colder seasons (Fig.2 to Fig.8). Although many authors describe these cyanobacteria as eurythermal organisms (WATERBURY *et al.*, 1986; SHAPIRO & HAUGEN, 1988; NEUER, 1992), the seasonal distribution of *Synechococcus* in the north-western Mediterranean Sea (AGAWIN *et al.*, 1998) and the northern Adriatic Sea (FUKS *et al.*, 2005) have shown an increased abundance of this genus during the warmer seasons and a lower abundance during the colder seasons.

In the coastal areas *Synechococcus* was distributed more or less uniformly in the water

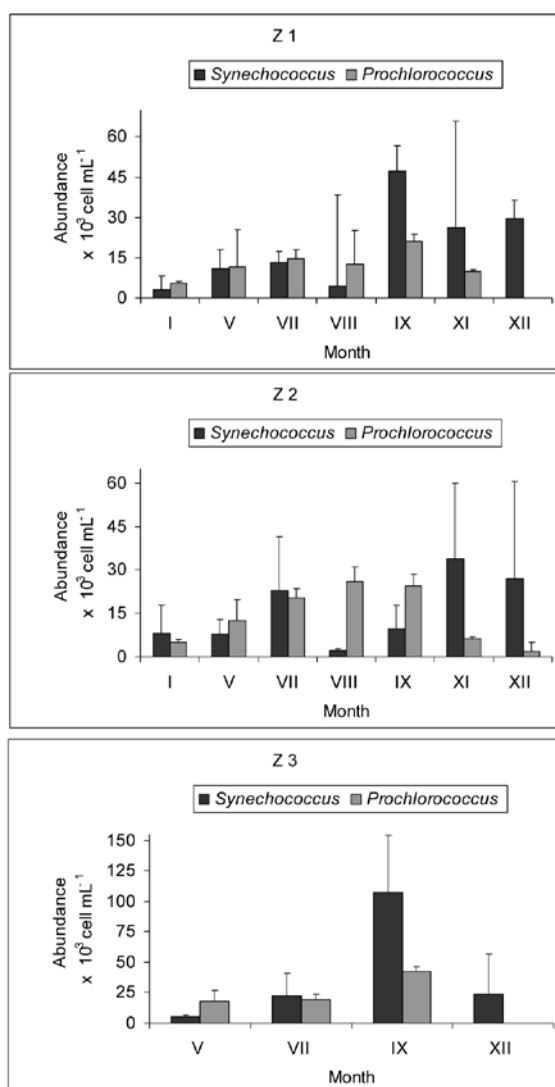


Fig. 2. Seasonal fluctuations of *Synechococcus* and *Prochlorococcus* in the area of Zadar. Average values (column) and positive standard deviation (bars) are presented

column. However, an uneven vertical distribution of *Synechococcus* was observed for the open sea area.

It is important to point out that at deep open sea stations; the abundance of *Synechococcus* was high in the bottom layer (Fig. 8), which agrees with the results of UYSAL & KÖKSALAN (2006) for the Mediterranean Sea and BERNARDI AUBRY *et al.* (2006) for the northern Adriatic Sea.

Therefore, high abundances of *Synechococcus* at the bottom layer is consistent with the finding that *Synechococcus* can successfully live in environments with limited light (WATERBURY

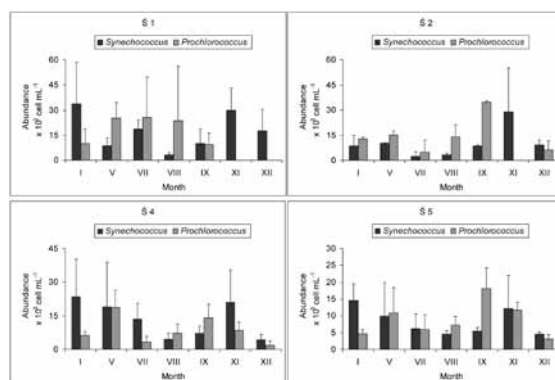


Fig. 3. Seasonal fluctuations of *Synechococcus* and *Prochlorococcus* in the area of Šibenik. Average values (column) and positive standard deviation (bars) are presented

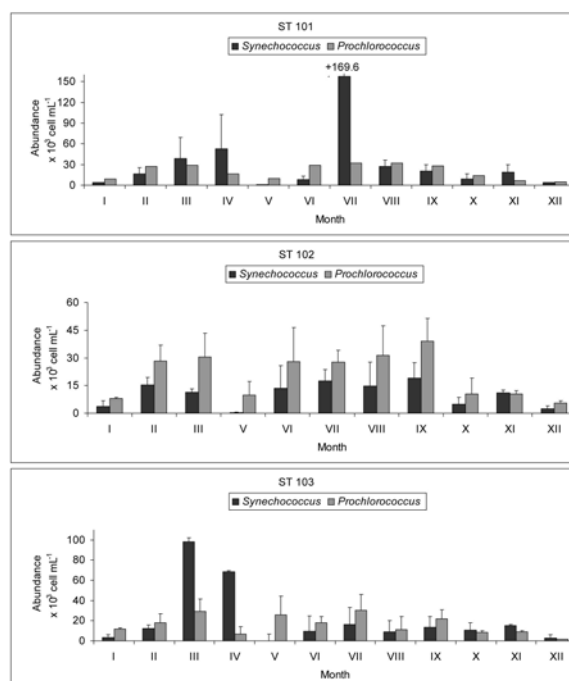


Fig. 4. Seasonal fluctuations of *Synechococcus* and *Prochlorococcus* in Kaštela Bay. Average values (column) and positive standard deviation (bars) are presented

*et al.*, 1986; WEHR, 1993) due to different pigment ecotypes (OLSON *et al.*, 1988).

Analysing the relationship between *Synechococcus* and dissolved inorganic nitrogen (DIN), the highest abundances of *Synechococcus* were found in the areas of Zadar and Kaštela Bay (Fig. 9), where concentrations of DIN were greater than  $1 \mu\text{mol L}^{-1}$ . In contrast, results obtained by VILIČIĆ *et al.* (2009) showed a

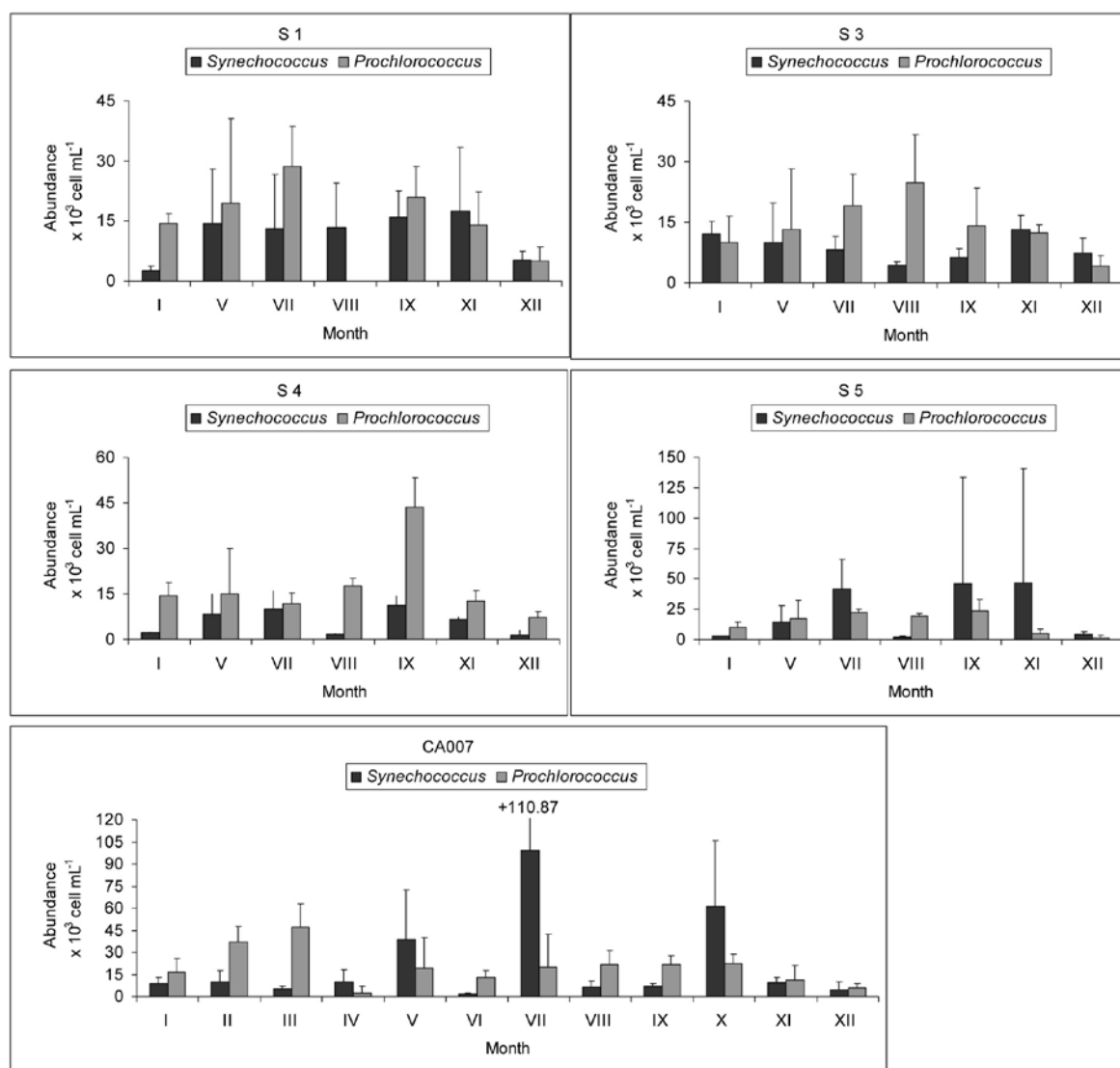


Fig. 5. Seasonal fluctuations of *Synechococcus* and *Prochlorococcus* in the area of Split. Average values (column) and positive standard deviation (bars) are presented

significant contribution of cyanobacteria in the DIN depleted environment of the Velebit area (less than  $0.5 \mu\text{mol L}^{-1}$ ). According to the literature data, *Synechococcus* mainly inhabits coastal mesotrophic and eutrophic systems in the north-western and eastern Mediterranean Sea (VAULOT *et al.*, 1990; UYSAL & KÖKSALAN, 2006). However, *Synechococcus* has also been shown to reach its highest abundance in the oligotrophic open sea area of the Mediterranean (VAULOT & PARTENSKY, 1992; MARTIN, 1997). The high abundance of *Synechococcus* in oligotrophic systems supports the suggestion that, by virtue of their small size,

they are more efficient in nutrient acquisition in oligotrophic environments than larger cells (RAVEN, 1986).

### Abundance of *Prochlorococcus*

The average cell abundance of *Prochlorococcus* in the central and southern coastal area ranged from 0 to  $4.7 \times 10^4 \text{ cells mL}^{-1}$ , while the average monthly abundance ranged from  $2.1 \times 10^3$  to  $1.1 \times 10^4 \text{ cells mL}^{-1}$  in the open sea. Like *Synechococcus*, variations in the abundances of *Prochlorococcus* were more pronounced in

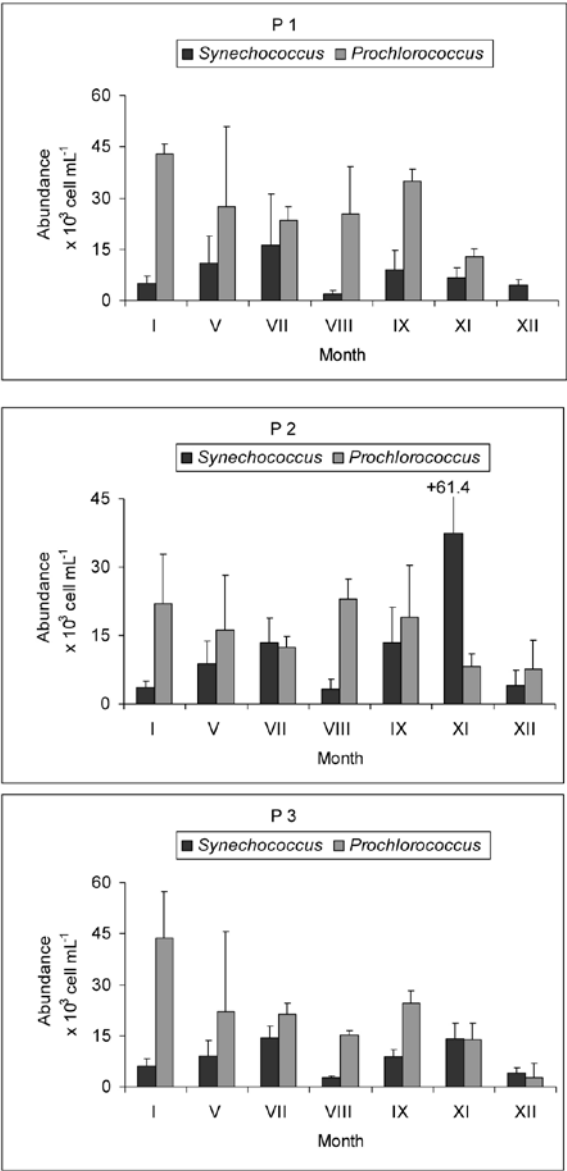


Fig. 6. Seasonal fluctuations of *Synechococcus* and *Prochlorococcus* in the area of Ploče. Average values (column) and positive standard deviation (bars) are presented

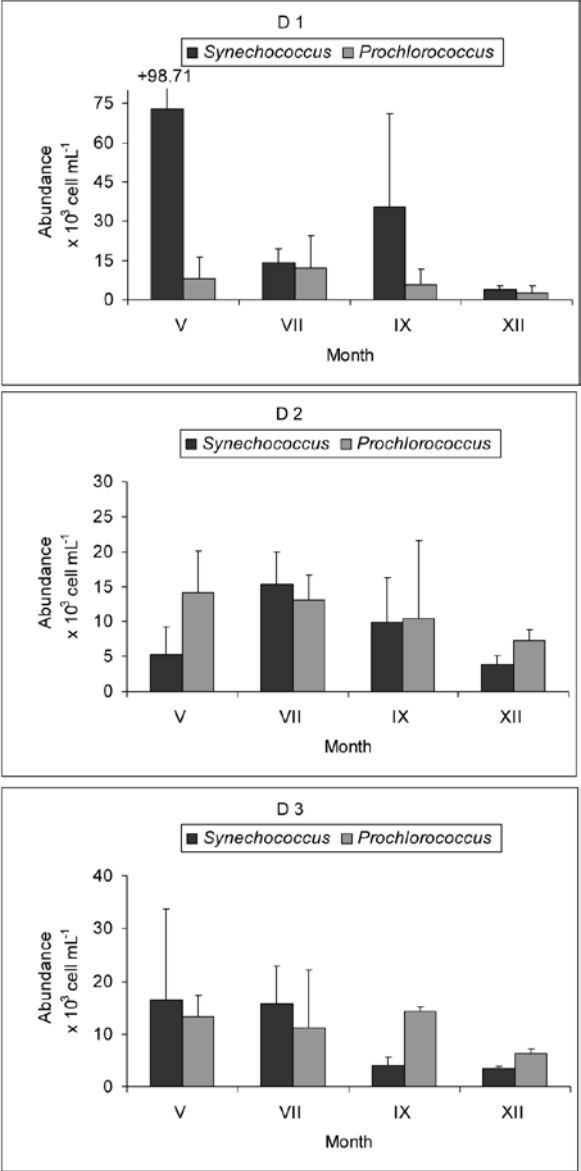


Fig. 7. Seasonal fluctuations of *Synechococcus* and *Prochlorococcus* in the area of Dubrovnik. Average values (column) and positive standard deviation (bars) are presented

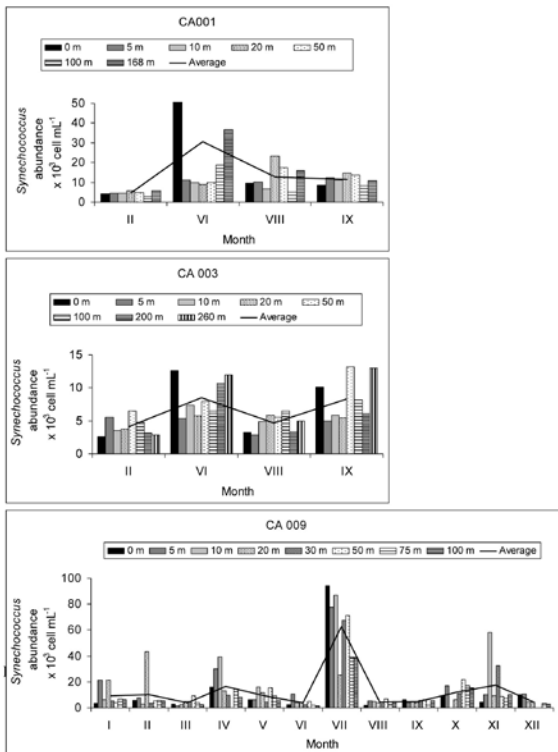


Fig. 8. Seasonal fluctuations of *Synechococcus* in the open sea area

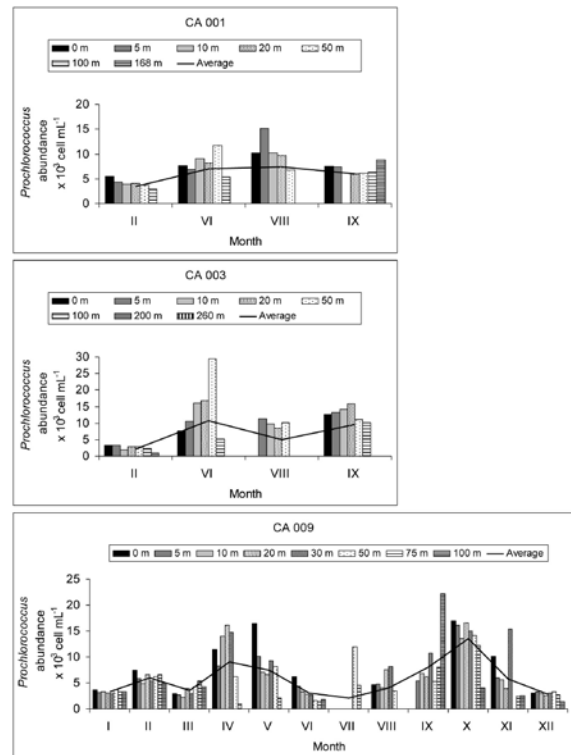


Fig. 10. Seasonal fluctuations of *Prochlorococcus* in the open sea area

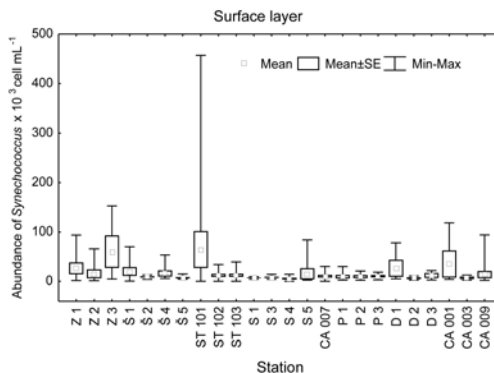


Fig. 9. Abundance of *Synechococcus* in the surface layer

the coastal areas compared to the open sea area (Fig. 2 to Fig. 7; Fig.10). Comparing all investigated areas of various depths to the values of the surface layer, the highest individual number of *Prochlorococcus* was found at the station located at the mouth of the river Krka (Š 1) and was  $7.1 \times 10^4$  cells  $\text{mL}^{-1}$  (Fig. 11). This is consistent with the high abundance of *Prochlorococcus* recorded in the Mediterranean coastal and open sea waters, where abundances were shown to range, in average order of magnitude, from  $10^3$  to  $10^4$  cells  $\text{mL}^{-1}$  (SOMMARUGA *et al.*, 2005; GARCZAREK *et al.*, 2007).

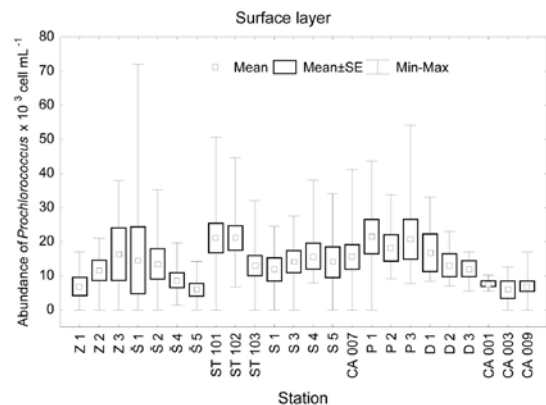


Fig. 11. Abundance of *Prochlorococcus* in the surface layer

In this study, high concentrations of *Prochlorococcus* were found during the warmer seasons at most of the coastal (Fig. 2 to Fig.7) and open sea areas (Fig. 10), while high *Prochlorococcus* abundance was observed in both the summer and winter in the Ploče area (Fig. 6). In many previous papers, seasonal distribution with high abundances were recorded during the summer (PARTENSKY *et al.*, 1999a, b; PAN *et al.*, 2005; PAN *et al.*, 2007). However, research results presented



in this study indicate that *Prochlorococcus* was detectable within the temperature range of 6.33 °C to 26.93 °C (Table 1), similar to some reports for the northern Atlantic and north-western Mediterranean Sea (BUCK *et al.*, 1996; AGAWIN *et al.*, 2000). In this study, the presence of *Prochlorococcus* cells in the water column was observed during all four seasons in coastal and open sea areas. This is consistent with the results reported by VAULOT *et al.* (1990) for the north-western Mediterranean (the mouth of the river Rhône), which noted the presence of this genus during the winter period, and with results from coastal systems, such as the coast of California or the Bay of Biscay where *Prochlorococcus* was seen to inhabit the water column from late winter to mid-summer (WORDEN *et al.*, 2004; CALVO-DÍAZ & MORÁN, 2006).

The maximum depth at which *Prochlorococcus* was found in this study was 200 metres at a station located in the Jabuka Pit, with an abundance of  $10^3$  cells mL<sup>-1</sup> in February when the water column was mixed (Fig. 10). Previous investigations have revealed high *Prochlorococcus* abundance in deeper layers of the euphotic zone (WEHR, 1993), even at depths of 150 to 200 metres (PARTENSKY *et al.*, 1999a). The most likely reason for their occurrence at this depth is vertical mixing of the water column (BERNARDI AUBRY *et al.*, 2006) or perhaps the existence of two *Prochlorococcus* ecotypes that inhabit the shallow and deeper euphotic layer (MOORE *et al.*, 1998; PARTENSKY *et al.*, 1999a).

Analysing the relationship between *Prochlorococcus* and nutrient availability in the coastal study area did not reveal any correlation between *Prochlorococcus* abundance and nutrient concentration. This is corroborated by CROSBIE & FURNAS (2001), which supports the theory that this genus may inhabit environments with different nutrient concentrations probably as a consequence of the existence of multiple ecotypes in the marine environment (VAULOT *et al.*, 1990). On the other hand, a negative correlation between the abundance of this genus and availability of nitrate was found in the open sea during this study (Fig. 12). This can be explained by the fact that increases in nitrate concentrations do not support the growth of *Prochlorococcus* because

they do not possess the nitrate reductase enzyme (MOORE *et al.*, 2002; LOPEZ-LOZANO *et al.*, 2002). It is also important to highlight the fact that during the investigated period, the deep waters in the eastern middle Adriatic Sea were influenced by the warmer and more saline Levantine Intermediate Water which brings higher concentrations of nitrate (ŠOLIĆ *et al.*, 2008).

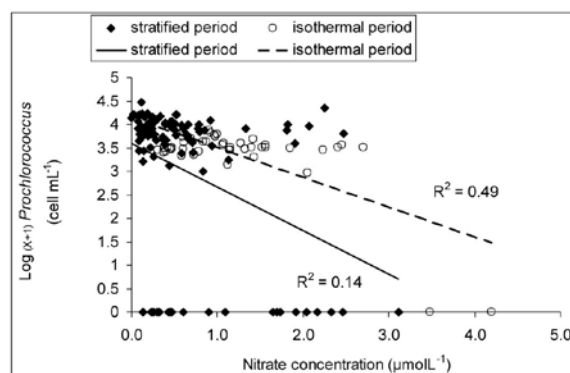


Fig. 12. Relationship between concentration of nitrates and *Prochlorococcus* abundance during the isothermal and stratified periods

Besides abiotic controlling factors such as nutrient availability and temperature, cyanobacteria are also influenced by biotic controlling factors such as grazing by heterotrophic nanoflagellates (HNF). Many authors have found that HNF are important grazers of *Prochlorococcus* (GUILLOU *et al.*, 2001; CHRISTAKI *et al.*, 2001; CHRISTAKI *et al.*, 2002). A statistically significant positive correlation between *Prochlorococcus* abundance and HNF was found for the open sea during the isothermal period of the water column (Fig. 13), which indicates the consumption of these cells by HNF.

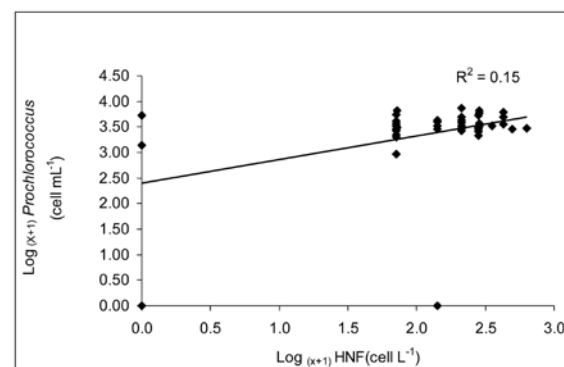


Fig. 13. Relationship between HNF and *Prochlorococcus* abundance during the isothermal period

## CONCLUSIONS

In this study, we determined the average number of *Synechococcus* to be in the range from  $10^2$  to  $10^5$  cells mL<sup>-1</sup> and *Prochlorococcus* in the range from 0 to  $10^4$  cells mL<sup>-1</sup>. The vertical distribution at the open sea stations showed high values of *Synechococcus* and *Prochlorococcus* abundance in deeper layers. *Synechococcus* was found to be more abundant than *Prochlorococcus* in most cases. The abundance of *Synechococcus* in the Adriatic Sea was influenced more by temperature than nutrient availability, while the abundance of *Prochlorococcus* was influenced by nutrient availability as a consequence of the movement of water mass, temperature and HNF, respectively.

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## Rasprostranjenost rodova *Synechococcus* i *Prochlorococcus* u srednjem Jadranu

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### SAŽETAK

Brojnosti rodova *Synechococcus* i *Prochlorococcus* određene su na dvadeset i jednoj postaji smještenoj na području priobalnog mora srednjeg i južnog Jadrana te na tri postaje smještene na području pučinskog mora srednjeg Jadrana. Brojnost roda *Synechococcus* iznosila je u rasponu od  $10^2$  do  $10^5$  st  $\text{mL}^{-1}$  na priobalnom području, te od  $10^3$  do  $10^4$  st  $\text{mL}^{-1}$  na području pučinskog mora. Brojnost roda *Prochlorococcus* iznosila je u rasponu od 0 do  $10^4$  st  $\text{mL}^{-1}$  na priobalnom području, te od  $10^3$  do  $10^4$  st  $\text{mL}^{-1}$  na području pučinskog mora. Sezonska raspodjela brojnosti navedenih rodova uglavnom je pokazala porast vrijednosti u toplijem te smanjenje vrijednosti u hladnijem razdoblju. Najviša brojnost *Synechococcus* utvrđena je u Kaštelanskom zaljevu i iznosila je  $4.6 \cdot 10^5$  st  $\text{mL}^{-1}$ , dok je najviša vrijednost *Prochlorococcus* utvrđena na području Šibenika te je iznosila  $7.1 \times 10^4$  st  $\text{mL}^{-1}$ . Rezultati istraživanja pokazali su uglavnom prevladavanje brojnosti roda *Synechococcus* nad brojnostima roda *Prochlorococcus*. Temperatura je imala veći utjecaj na promjene brojnosti roda *Synechococcus* u odnosu na raspoložive koncentracije hranjivih soli. Rod *Prochlorococcus* bio je pod većim utjecajem raspoloživih koncentracija hranjivih soli i gibanja vodenih masa u odnosu na utjecaj HNF-a.

**Ključne riječi:** *Synechococcus*, *Prochlorococcus*, Jadransko more

